Supplemental data for the manuscript

The type I BMP receptor, Alk3, is required for the induction of hepatic hepcidin gene expression by interleukin-6

Claire Mayeur[1], Lisa K. Lohmeyer[#1], Patricio Leyton[1], Sonya M. Kao[1], Alexandra E. Pappas[1], Starsha A Kolodziej[1], Ester Spagnolli[1], Binglan Yu[1], Rita L. Galdos[2], Paul B. Yu[3,4], Randall T. Peterson[3], Donald B. Bloch[1,2], Kenneth D. Bloch*[1,3], and Andrea U. Steinbicker*[1,5]

[1]Anesthesia Center for Critical Care Research of the Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA

[2]Rheumatology, Allergy, and Immunology Division and [3]Cardiovascular Research Center of the Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA

[4]Cardiovascular Division, Brigham and Women`s Hospital, Boston, MA 02115, USA

[5]Department of Anesthesiology, Intensive Care and Pain Medicine, University Hospital Muenster, University of Muenster, 48149 Muenster, Germany

[#]Current address: Clinic for Hematology, Oncology and Stem Cell Transplantation, University Medical Center Freiburg, 79095 Freiburg, Germany

*Drs. Bloch and Steinbicker contributed equally to the manuscript.

Running title: Alk3 and interleukin-6 mediated hepcidin induction

Correspondence should be addressed to:
Andrea U. Steinbicker, MD, MPH
Department of Anesthesiology, Intensive Care and Pain Medicine
University Hospital Muenster
Albert-Schweitzer Campus 1, Gebäude A1
48149 Muenster
Germany
Phone: 0049-251-83-47898
Fax: 0049-251-83-48667
Email: andrea.steinbicker@ukmuenster.de, andrea.steinbicker@gmail.com
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Supplemental Table

Table S1.

Sybr Primers:

18S-F: 5'-CGGCTACCACTCCAAGGAA-3'

18S-R: 5'-GCTGGAATTACCGCGGCT-3'

hepcidin-F: 5`-CTGAGCAGCACCACCTATCTC-3`

hepcidin-R: 5`-TGGCTCTAGGCTATGTTTTGC-3`

HO-1-F: 5'-AAGCCGAGAATGCTGAGTTCA-3'

HO-1-R: 5'-GCCGTGTAGATATGGTACAAGGA-3'

Taqman primers:

18S: Hs99999901_s1 18S FAM

Hepcidin: Mm00519025_m1 Hamp

Alk2: Mm00431646_m1 Acvr1 FAM

Alk3: Bmpr1a- Mm00477650_m1 Bmpr1a

Id-1: Mm00775963_g1 Id1

Table S1. Sequences of Sybr primers, and Life technologies assay IDs of Taqman primers used for measuring mRNA levels with qRT-PCR.

Supplemental Figures

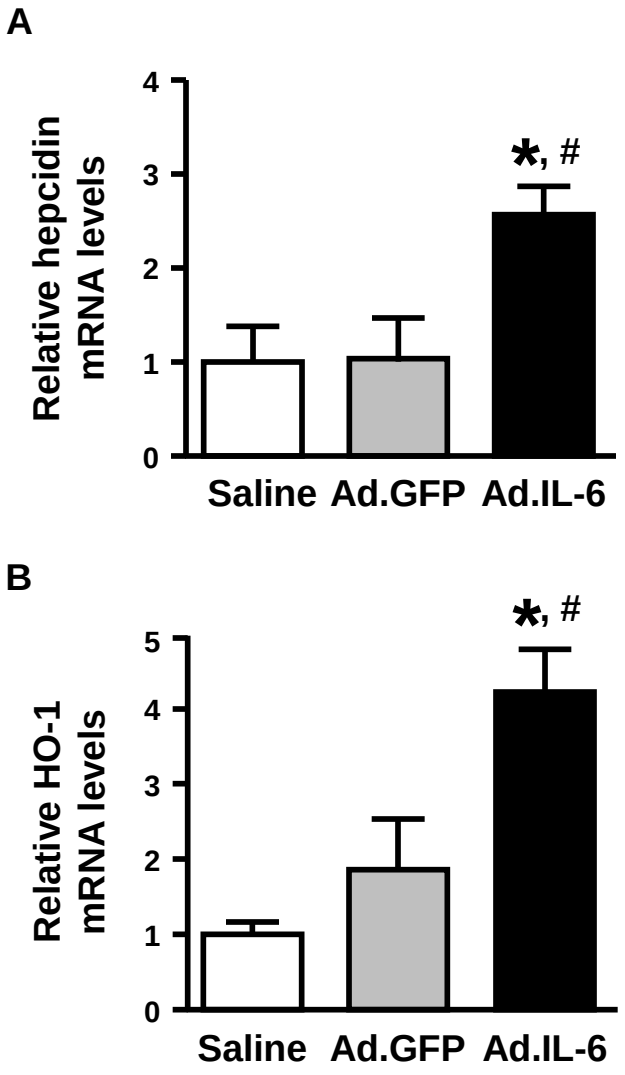
Figure S1. Hepatic hepcidin and HO-1 mRNA levels in mice injected with saline, Ad.GFP, or Ad.IL-6. Eight-week-old C57Bl/6 male mice received an intravenous injection of saline (100 µl), an adenovirus specifying green fluorescent protein (Ad.GFP, $1.5x10^{11}$ virus particles), or an adenovirus specifying interleukin 6 (Ad.IL-6, $1.5x10^{11}$ virus particles) (n=3, in each group). Mice were sacrificed 72 hours after injection. (**A**) Hepatic hepcidin mRNA levels (1 way-ANOVA, p=0.0034; *p=0.0068: mice injected with saline vs mice injected with Ad.IL-6; $^{\#}$p=0.0079: mice injected with Ad.GFP vs mice injected with Ad.IL-6). (**B**) Hepatic HO-1 mRNA levels (1 way-ANOVA, p=0.0004; *p=0.001: mice injected with saline vs mice injected with Ad.IL-6; $^{\#}$p=0.0034: mice injected with Ad.GFP vs mice injected with Ad.IL-6).

Figure S2. Hepatic hepcidin and serum iron levels in C57Bl/6 mice injected with murine IL-6 (mIL-6). Ten-week-old C57Bl/6 male mice were injected intraperitoneally with either mIL-6 (100 ng/g) or vehicle (0.1% BSA in PBS, 10 µl/g, n=3-4 in each group). Two and 4 hours after injection, mice were sacrificed, blood was collected, and livers were harvested. (**A**) Hepatic hepcidin mRNA levels ($^{\#}$p<0.02: mice injected with mIL-6 vs vehicle, after 4 hours). (**B**) Serum iron levels.

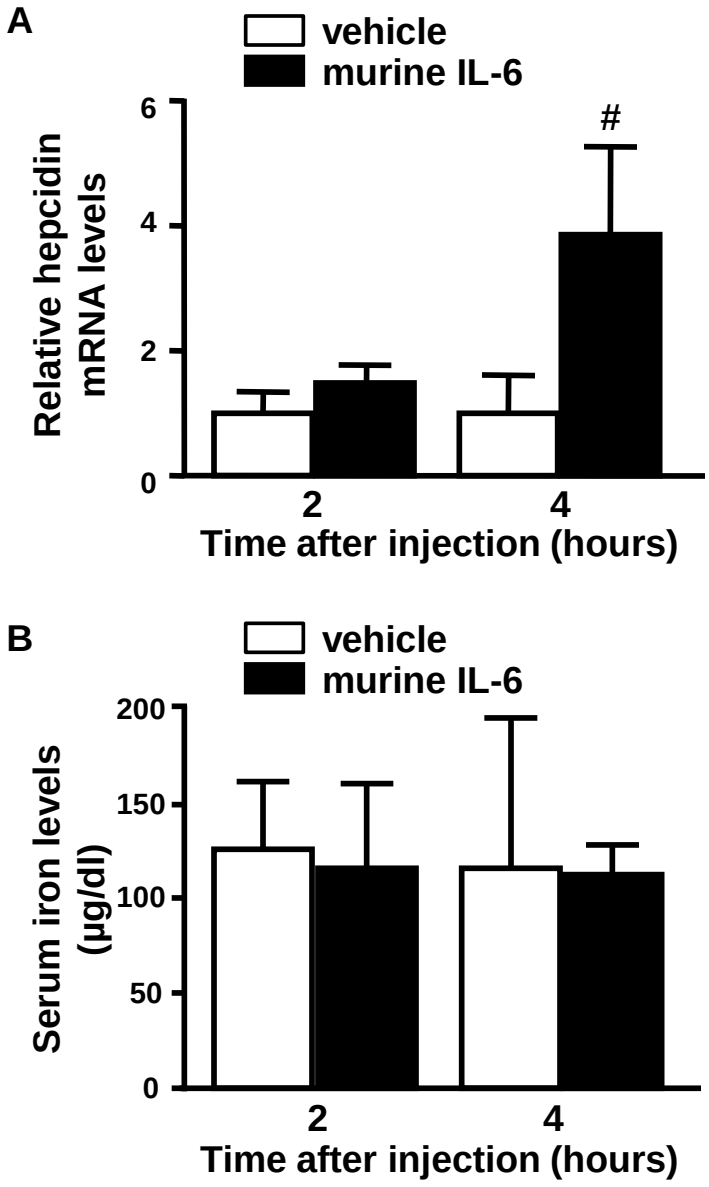
Figure S3. Liver and spleen iron content in mice injected with murine IL-6 or vehicle. Four hours after intraperitoneal injection of mIL-6 or vehicle, mice were sacrificed, and liver and spleen were harvested. (**A**) Liver iron content (LIC) of *Alk2*$^{fl/fl}$ and *Alk2*$^{fl/fl}$; *Alb-Cre* mice injected with mIL-6 or vehicle (1 way-ANOVA, p<0.0001; *p=0.04: *Alk2*$^{fl/fl}$ mice injected with vehicle vs *Alk2*$^{fl/fl}$*;Alb-Cre* mice injected with vehicle; $^{\#}$p=0.004: *Alk2*$^{fl/fl}$ mice injected with mIL-6 vs *Alk2*$^{fl/fl}$*;Alb-Cre* mice injected

with mIL-6). (**B**) Splenic iron content (SIC) of *Alk2*$^{fl/fl}$ and *Alk2*$^{fl/fl}$; *Alb-Cre* mice injected with mIL-6 or vehicle. (**C**) LIC of *Alk3*$^{fl/fl}$ and *Alk3*$^{fl/fl}$; *Alb-Cre* mice injected with mIL-6 or vehicle (1 way-ANOVA, p<0.0001; *p<0.002: *Alk3*$^{fl/fl}$ mice injected with vehicle vs *Alk3*$^{fl/fl}$*; Alb-Cre* mice injected with vehicle; $^{\#}$p=0.0006: *Alk3*$^{fl/fl}$ mice injected with mIL-6 vs *Alk3*$^{fl/fl}$*; Alb-Cre* mice injected mIL-6). (**D**) SIC of *Alk3*$^{fl/fl}$ and *Alk3*$^{fl/fl}$; *Alb-Cre* mice injected with mIL-6 or vehicle (1 way-ANOVA, p=0.0009; *p=0.012: *Alk3*$^{fl/fl}$ mice injected with vehicle vs *Alk3*$^{fl/fl}$*; Alb-Cre* mice injected vehicle; $^{\#}$p=0.0025: *Alk3*$^{fl/fl}$ mice injected with mIL-6 vs *Alk3*$^{fl/fl}$*; Alb-Cre* mice injected with mIL-6).

Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3